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Technical Report

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Botulinum Antitoxin as a Therapeutic Agent in Monkeys With Experimental Botulism

by

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FOREWORD

The work described in this report was authorized under Project 1C622401A097, Medical Defense Aspects of Chemical Agents (U). The experimental data are contained in notebooks MN-1687, MN-1703, and MN-1722. This work was started in January 1963 and completed in August 1964.

In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

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Notices

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

Disposition

When this report has served its purpose, DESTROY it.

DIGEST

Antitoxin administered soon after the appearance of the first toxic signs in monkeys given 2.5 to 5.0 LD50's of botulinum toxin resulted in complete recovery in 11 out of 15 animals when liquid food and fluids were forced during the period of aphagia. When no supportive treatment was applied, only one out of six survived. The survivor had received 2.5 LD50's of toxin.

It is concluded that most monkeys given 2.5 to 5.0 LD50's of botulinum toxin intravenously can be saved when the antitoxin is administered soon after the first toxic signs are detected and when daily, supplemental, intragastric feedings, vitamins, and antibiotics are given.

CONTENTS

BOTULINUM ANTITOXIN AS A THERAPEUTIC AGENT IN MONKEYS WITH EXPERIMENTAL BOTULISM

I. INTRODUCTION.

Botulinum antitoxin will prevent the occurrence of toxic signs and death when given to a nonimmunized animal sufficiently early after a lethal dose of botulinum toxin has been administered. A diagnosis of botulism in humans accidentally poisoned by eating food containing the toxin, however, probably would not be made until after toxic signs and symptoms had appeared. It is unlikely, therefore, that antitoxin therapy would be begun before diagnosis. The critical time for administering antitoxin has not yet been defined.

In the present study, monkeys were administered intravenous doses of botulinum toxin followed by antitoxin after early toxic signs were observed. Observations were made for toxic signs and for the effectiveness of the antitoxin, with and without supportive treatment, as a therapeutic agent for lifesaving.

II. EXPERIMENTATION.

A. Materials.

1. Animals.

Thirty-five sooty mangabey and rhesus monkeys, weighing 2 to 6 kg, were used.

2. Agent.

The botulinum toxin, type A, was similar to that described by Lamanna, McElroy, and Eklund.* The mouse intraperitoneal LD50 (MU) for mice weighing 25 gm for this partially purified toxin was approximately 3×10^{-4} µg. The stock solution was prepared from the powdered material dissolved in a sterile gelatin-phosphate buffer solution (10 gm Na_2HPO_4 and 3 gm Difco gelatin in 1 ℓ distilled water); pH was adjusted to 6.8 by the addition of concentrated HCl. All stock solutions were bioassayed in mice for potency before they were released for use in these studies. The potency of the diluted solutions ranged from 100 to 1,000 MU/ml.

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* Lamanna, C., McElroy, O. E., and Eklund, H. W. The Purification and Crystallization of Clostridium Botulinum Type A Toxin. Science 103, 613-614 (1946).

3. Antitoxin.

Bivalent botulinum antitoxin (equine origin), globulin-modified, 500 units/ml each of types A and B (Lederle), was used for the antitoxin.

B. Procedure.

Monkeys received the botulinum toxin intravenously in doses varying from 2.5 to 5.0 LD50's. These doses were based on the finding of Herrero and coworkers* that the intravenous LD50 of this toxin for the rhesus monkey is 40 MU/kg. Times to occurrence of the various toxic signs (ptosis of the eyelids, weakness, increased salivation, dyspnea, and collapse) were recorded. Not all animals were observed continuously during the night; therefore, these data are sometimes incomplete. Time to death was recorded as accurately as possible.

The 35 monkeys were divided into four groups and after receiving the toxin were treated as follows:

1. Fourteen controls received no treatment of any kind.

2. Six animals received only antitoxin.

3. Five animals received antitoxin and, 72 hr after administration of toxin, supplemental feeding and fluids.

4. Ten animals received antitoxin and, starting 48 hr after administration of toxin, daily supplemental feeding and fluids.

When antitoxin (1,000 to 2,700 units/animal) was used in the therapy, it was administered soon after the first toxic signs became evident, except for animal no. 70, who received antitoxin when signs were severe. The group that did not receive supportive treatment until at least 72 hr after the toxin was fed as follows:

* Herrero, Brunildo, A., Ecklund, Allen E., Street, C. Spencer, Ford, Duane F., and King, John K. CRDLR 3235. Experimental Botulism in Monkeys. November 1964. UNCLASSIFIED Report.

One animal.	72 hr:	50 ml of saline by stomach tube
One animal.	75 hr:	100 ml of saline subcutaneously
	95 hr:	100 ml of milk mix* by stomach tube; 50 to 75 ml of saline subcutaneously
	143 hr:	100 ml of milk mix by stomach tube
	167 hr:	100 ml of milk mix by stomach tube and 75 ml of saline subcutaneously
	191 hr:	100 ml of milk mix by stomach tube and 100 ml of saline subcutaneously
Two animals.	75 hr:	100 ml of saline subcutaneously
	95 hr:	100 ml of milk mix by stomach tube; 50 to 75 ml of saline subcutaneously
	143 hr:	100 ml of milk mix by stomach tube
One animal.	eighth day:	60 ml of Dextran, 6% by intravenous drip, and 40 ml of 5% dextrose subcutaneously

The group that received a more ordered regimen of daily supportive treatment, usually starting about 48 hr after administration of the toxin, was given 50 ml of milk mix* containing 1 ml of vitamins ** intragastrically, 4 ml of antibiotics† intramuscularly, and 200 to 250 ml of 5% dextrose intraperitoneally.

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* Milk mix: Sobee powder milk mix in water according to directions. Sobee powder is made by Mead Johnson and Co, Evansville, Indiana. In normal dilution (w/v), it contains the following ingredients: protein, 3.2%; fat, 2.6%; carbohydrate, 7.7%; crude fiber, 0.2%; minerals (ash), 0.5%; iron, 0.0005%; and moisture, 85.8%; 100 gm supplies 465 cal.

** Octavitamin drops made by Success Chemical Co., Inc., Brooklyn, New York.

† Combiotic: Each 2 ml contains 400,000 units of penicillin G procaine crystalline and 0.5 gm dihydrostreptomycin as the sulfate with 2% procaine HCl and preservatives (Pfizer Laboratories, Division of Charles Pfizer and Co., Inc., New York, New York).

III. RESULTS.

Table 1 lists the times to first occurrence of certain toxic signs and times to death of untreated control animals. No animal survived. The times to death varied between 32 and 135 hr after administration of the toxin. The times to first occurrence of toxic signs varied between 20 and 38 hr.

The animals listed in table 2 received only antitoxin. There was one survivor among the six animals. The times to occurrence of toxic signs and to death were within the same range as those for the controls.

The animals listed in table 3 received antitoxin but no supportive treatment until 72 hr after administration of the toxin. The supportive treatment was given soon after the animal showed inability to eat and drink. One animal (no. 30, 2.5 LD50's of toxin) ate and drank during the first 7 days, but was unable to do so on the eighth and ninth days. Dextran intravenously and saline subcutaneously were administered on the eighth day. From the tenth day on, the animal was able to eat and drink. Two of the five animals in this group died. The times to death were somewhat later than was the latest death among the animals treated only with antitoxin.

In table 4 are listed the results for 10 animals given daily supportive treatment beginning shortly after the antitoxin was administered. There were two deaths. One of these (no. 70) received the antitoxin when the toxic signs were severe. This monkey collapsed immediately after the antitoxin injection and remained in a weakened condition until death 20 hr later. The other animal (no. 74) at first appeared to be in a satisfactory condition; later, it was seen several times lying on its side in the cage, but was easily aroused and could move around. This animal died after a routine feeding 48-1/2 hr after administration of the antitoxin. Necropsy revealed acute peritonitis.

IV. DISCUSSION.

There is ample information available in the literature showing that botulinum antitoxin will prevent death in animals when administered soon after the toxin and before the appearance of toxic signs. In mice receiving 80 LD50's of toxin intravenously, the critical time for administering antitoxin is shortly before the appearance of toxic signs.* Antitoxin injected

* Crook, J. W., Cresthull, P., and Oberst, F. W. CRDLR (in preparation). The Effectiveness of Bivalent Botulinum Antitoxin and Drug Therapy Against Clostridium Botulinum Type A Toxin in Mice.

TABLE 1

TIMES TO OCCURRENCE OF SIGNS AND DEATH IN MONKEYS GIVEN BOTULINUM TOXIN (CONTROLS)

Animal number	Toxin dose	Times to occurrence of toxic signs					Times to death
		Eyelid ptosis	Muscular weakness	Increased salivation	Dyspnea	Collapse	
	LD50	hr					
45	5 0	-	-	-	-	-	43
51	5.0	28	< 46.5	< 46.5	-	-	64
60	5.0	28.6	28.6	-	-	-	49
64	5.0	34.4	34.4	-	-	-	73
17	4.6	29	34	33	34	-	38
19	3.75	24	27.5	25.6	-	31.5	32
20	3.75	20.3	24	23	-	38	38
23	3.75	34	39	38	39	-	49
29	2.5	< 38.4	< 38.4	-	-	-	40
32	2.5	-	-	-	-	-	135
33	2.5	-	-	39.3	39.3	-	49
35	2.5	45	45	38	-	-	130
40	2.5	38-45	-	-	-	46-51	67
41	2.5	45.5	-	38.2	-	-	67

Note: A blank space (-) means only that time to occurrence was not recorded.

TABLE 2

TIMES TO OCCURRENCE OF SIGNS AND DEATH IN MONKEYS GIVEN BOTULINUM TOXIN AND TREATED WITH ANTITOXIN

Animal number	Toxin dose	Times to antitoxin	Times to occurrence of first toxic signs				Times to death
			Eyelid ptosis	Muscular weakness	Salivation	Dyspnea	
	LD50	hr					
16	4.6	29.0	29.0	29.0	29.0	29.0	38
28	2.5	37.3	39.0	39.0	37.3	-	135
31	2.5	35.5	41.5	47.5	35.5	-	134
34	2.5	38.6	38.6	38.6	37.1	-	134
36	2.5	43.0	42.4	38.7	-	-	Survived
37	2.5	38.9	38.9	48.5	47.4	38.9	98

Note: A blank space (-) means only that time to occurrence was not recorded.

TABLE 3

THERAPEUTIC EFFECT OF ANTITOXIN AND SUPPORTIVE TREATMENT (BEGINNING 72 HR POSTEXPOSURE) ON MONKEYS WITH BOTULISM

Animal number	Toxin dose	Times to antitoxin	Times to occurrence of first toxic signs			Times to death	Nature of supportive treatment*
			Eyelid ptosis	Muscular weakness	Salivation		
	LD50	hr					
49	5.0	25.3	25.3	25.3	None	Survived	A
18	3.75	28.0	36.0	36.0	28.0	216	B
21	3.75	23.2	23.0	31.0	27.0	141	C
22	3.75	31.1	31.1	44.0	-	Survived	C
30	2.5	38.2	38.2	38.2	-	Survived	D

* After toxin administration:

A. 72 hr: 50 ml of saline by stomach tube

B. 75 hr: 100 ml of saline subcutaneously
95 hr: 100 ml of milk mix by stomach tube; 50 to 75 ml of saline subcutaneously
143 hr: 100 ml of milk mix by stomach tube
167 hr: 100 ml of milk mix by stomach tube and 75 ml of saline subcutaneously
191 hr: 100 ml of milk mix by stomach tube and 100 ml of saline subcutaneously

C. 75 hr: 100 ml of saline subcutaneously
95 hr: 100 ml of milk mix by stomach tube; 50 to 75 ml of saline subcutaneously
143 hr: 100 ml of milk mix by stomach tube

D. eighth day: 60 ml of Dextran, 6% by intravenous drip, and 40 ml of 5% dextrose subcutaneously

Note: A blank space (-) means only that time to occurrence was not recorded.

TABLE 4

THERAPEUTIC EFFECT OF ANTITOXIN AND SUPPORTIVE TREATMENT (BEGINNING 24 HR POSTEXPOSURE) ON MONKEYS WITH BOTULISM

Animal number	Toxin dose	Times to antitoxin	Times to occurrence of first toxic signs		Times to death
			Ptosis	Muscular weakness	
	LD50	hr			
59	5.0	29.1	28.3	27.3	Survived
66	5.0	31.6	31.6	30.6	Survived
67	4.9	29.5	27.8	27.8	Survived
68	4.9	29.5	27.7	27.7	Survived
69	4.9	30.4	28.9	28.9	Survived
70	4.9	29.6	27.5	27.5	49.5
71	4.0	33.9	27.5	31.9	Survived
72	4.0	34.2	33.7	27.8	Survived
73	4.0	28.7	28.5	26.5	Survived
74	4.0	28.2	26.6	26.6	76.7

into the bloodstream of dogs neutralizes all the uncombined toxin in the circulation.* At present, there is no evidence that the portion of the toxin already fixed by the tissues is affected by antitoxin. In the present study, it was demonstrated (table 4) that at least 80% of monkeys given 4.0 to 5.0 LD50's of toxin intravenously can be saved, provided that the antitoxin is administered soon after the appearance of the first signs and that suitable nursing care is provided during the period of aphagia. One of the two animals that died might have survived had it not developed peritonitis; the other animal appeared to be quite ill, more than the others, when the antitoxin was given.

V. CONCLUSION.

Antitoxin administered soon after the appearance of the first toxic signs in monkeys given 2.5 to 5.0 LD50's of botulinum toxin resulted in complete recovery in 11 out of 15 animals when liquid food and fluids were forced during the period of aphagia. When no supportive treatment was applied, only one out of six survived. The survivor had received 2.5 LD 50's of toxin.

It is concluded that most monkeys given 2.5 to 5.0 LD50's of botulinum toxin intravenously can be saved when the antitoxin is administered soon after the first toxic signs are detected and when daily, supplemental, intragastric feedings, vitamins, and antobiotics are given.

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* House, Michael J., Cresthull, Paul, Crook, James W., and Oberst, Fred W. CRDLR 3229. Changes in Concentration of Botulinum Toxin in Dog Serum After Parenteral Administration. September 1964. UNCLASSIFIED Report.

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13 ABSTRACT

Botulinum antitoxin, types A and B, was administered irtravenously to monkeys after appearance of toxic signs resulting from administration of 2.5 to 5.0 LD50's of botulinum toxin. Recoveries were realized in 11 out of 15 animals when liquid food and fluids were forced during the aphagia period. Only one out of six monkeys survived when no supportive treatment was applied. The survivor had received only 2.5 LD50's of toxin. The intravenous administration of antitoxin soon after detection of toxic signs can save most monkeys. Daily intragastric feedings, vitamins, and antibiotics must be included in the therapeutic treatment.

14 KEYWORDS

Botulinum antitoxin, type A
Botulinum antitoxin, type B
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Therapeutic agent
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Rhesus monkeys
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